IN THE CLAIMS:

Please amend claims 1-3 and 21, as follows:

1. (Twice amended) A method for obtaining a transgenic embryo, comprising the steps of:

incubating an exogenous nucleic acid with a membrane-disrupted sperm head or a demembranated sperm head for a period of time;

co-inserting the exogenous nucleic acid and the sperm head into an unfertilized oocyte to form a transgenic fertilized oocyte; and

allowing the transgenic fertilized oocyte to develop into a transgenic embryo.

2. (Twice amended) The method of claim 1, wherein the co-inserting step is accomplished by piezo-electrically actuated microinjection.

3. (Twice amended) The method of claim 2, wherein the exogenous nucleic acid and the membrane-disrupted sperm head are co-inserted into the cytoplasm of the unfertilized oocyte.

21. (Twice amended) A method for obtaining a transgenic embryo, comprising the steps of:

obtaining a membrane-disrupted sperm head or a demembranated sperm head;

mixing the membrane-disrupted sperm head or demembranated sperm head with an exogenous nucleic acid;

co-inserting the mixture into an isolated unfertilized metaphase II oocyte to form a transgenic fertilized oocyte; and

allowing the transgenic fertilized oocyte to develop into a transgenic embryo.

REMARKS

Reconsideration of the above-reference application is respectfully requested. This is a response to the Office Action mailed September 26, 2000. The claim amendments herein are submitted under newly amended 37 CFR § 1.121(c), as published in the Official Gazette, September

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19, 2000. As required by the amended Rule, the clean copy of the amended claims is accompanied by the marked-up version attached hereto on a separate page.

By this Amendment, claims 1-3 and 21 have been amended to more clearly point out certain features of applicants' invention. No new matter has been added.

Claims 1-8 and 10-21 remain pending.

The Rejections Under 35 U.S.C. §103

In the Office Action, the Examiner rejected claims 1-8 and 10-21 under 35 U.S.C. 103(a) as being unpatentable over Lavitrano, taken with Kuretake, for the reasons of record, now adding claims 2 and 3 to the previous rejection of the claims.

The Examiner alleges that he has established that there is a correlation between fertilization by ICSI and transgenesis, because DNA transfer and, thus, transgenesis occurs at fertilization, meaning that without fertilization, transgenesis does not occur. The Examiner alleges that since both "live" and "dead" sperm are "functionally equivalent" in that both "are able to fertilize oocytes, the ability of both live and dead sperm to fertilize oocytes can be equated with the ability of both live and dead sperm to transfer DNA into an unfertilized oocyte to create a transgenic non-human animal while simultaneously fertilizing the same unfertilized oocyte." The Examiner then attempts to provide motivation for combining the Lavitrano and Kuretake references, alleging that "a means of increasing the rate of fertilization would also increase the rate of transgenesis, and that Kuretake discloses that sperm with a damaged plasma membrane increase the fertilization rate by ICSI."

Applicants traverse the rejections. For the reasons of record and the arguments presented below, applicants respectfully assert that neither Lavitrano nor Kuretake, alone or in combination, teach or suggest the present invention, as recited in the amended claims.

In particular, applicants respectfully note that Lavitrano suggests that "***only living sperm cells*** are able to take up DNA" (see page 717, column 2, last line of first paragraph under "Results"). This is in direct contrast to the present invention, in which the DNA associates with a clearly ***dead*** membrane-disrupted or demembranated sperm head (nucleus). Moreover, Lavitrano discloses that, after extensive washing of the live sperm cells, the remaining DNA was more stably

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associated with the spermatozoa and that "An attractive interpretation of these results would be that most or at least a portion of the DNA molecules associated with spermatozoa have penetrated into the heads of the sperm cells". (See page 717, column 2, last paragraph bridging to page 718, column 1, entire paragraph.) There is no question that Lavitrano is describing ***live, membrane-intact*** spermatozoa. Indeed other, later investigators have disclosed a model of the process through which DNA binds to ***live*** ejaculated spermatozoa and is internalized and penetrates the sperm nucleus. (See, *e.g.*, Spadafora, C. "Sperm cells and foreign DNA: a controversial relation", BioEssays 20:955-964, 1998, especially pages 957-959, Table 1 and Figure 3; Gandolfi, F. Sperm-Mediated Transgenesis, Theriogenology 53: 127-137, 2000, especially pages 128-130, paragraph 2. Copies of these references were previously submitted in an Information Disclosure Statement). In particular, the model teaches that, in order to associate with a sperm nucleus, the exogenous foreign DNA requires binding to a DNA-binding protein (DBP) located on ***the surface of the sperm plasma membrane***. The binding activates the mechanism of internalization of a ***DNA/DBP complex*** by association with CD4 molecules (on the surface of the sperm plasma membrane) to form a ***DNA/DBP/CD4*** complex ***which then penetrates the nucleus, followed by dissociation at the nuclear matrix, releasing the exogenous DNA in close contact with the sperm chromosomal DNA***. Applicants submit that each of these active events clearly requires a ***live*** spermatozoa having ***intact plasma membrane proteins*** that actively internalize the exogeous DNA, and that such proteins are not expected to be active in the dead, membrane-disrupted or demembranated spermatozoa of the present invention. Therefore, applicants respectfully assert that Lavitrano does not teach or suggest the present invention by which an exogenous nucleic acid is incubated with a ***dead*** membrane-disrupted or demembranated sperm head; neither does Lavitrano suggest that insertion of such nucleic acid and sperm head into an oocyte will result in the formation of a transgenic embryo and, optionally, a transgenic live offspring.

Applicants further assert that Kuretake does not remedy the defects of Lavitrano. In particular, Kuretake does not teach or suggest preincubation of ***dead*** membrane-disrupted or demembranated sperm heads with exogenous nucleic acid, let alone that ***transgenic*** embryos can be obtained by this method. Moreover, one of ordinary skill in the art would not be motivated to combine the teachings of Lavitrano and Kuretake with a reasonable expectation of success, because the entire prior art at the time of the present invention taught that ***live*** spermatozoa are necessary for

transferring exogenous nucleic acid to obtain transgenic embryos. Applicant asserts that the entire body of prior art teaches away from the present invention. Therefore, it would not have been obvious to combine the teachings of Lavitrano and Kuretake to arrive at the present invention.

Applicants further respectfully assert that the Examiner has misunderstood applicants' arguments in the Amendment filed July 11, 2000. In particular, applicants argued that the ability of whole ***live*** sperm cells to take up exogenous DNA has been demonstrated in a wide variety of animal species, and the exogenous DNA has been reported to become associated with the nucleus in the head of the ***live*** sperm. Because transgenesis using live sperm is not a species-specific phenomenon, one of ordinary skill in the art, upon reading the present specification, would not expect transgenesis using the present invention to be species specific. Notwithstanding, the prior art teaches away from employing dead spermatozoa and exogenous nucleic acid, rather than live spermatozoa and exogenous nucleic acid and, therefore, the uptake of exogenous nucleic acid with dead spermatozoa, and the use of such spermatozoa to produce transgenic embryos and/or offspring, is not obvious. Moreover, absent evidence to the contrary, the Examiner's argument equating the ability of live and dead sperm to fertilize oocytes with the ability of live and dead sperm to transfer exogenous DNA has no basis in the teachings of the prior art.

Withdrawal of the rejections under 35 U.S.C. 103(a) is respectfully requested.

Conclusion

In view of the foregoing amendments and Remarks, applicants submit that this application is in condition for allowance, and an early favorable response is respectfully solicited.

Respectfully submitted,

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U.S. Patent Application Serial No. 09/371,648
Claim Amendments
November 22, 2000

1. (Twice amended) A method for obtaining a transgenic embryo, comprising the steps of:

incubating an exogenous nucleic acid with a membrane-disrupted sperm head or a demembranated sperm head for a period of time [to obtain a sperm head comprising an associated exogenous nucleic acid];

[microinserting the sperm head comprising the associated exogenous nucleic acid] co-inserting the exogenous nucleic acid and the sperm head into an unfertilized oocyte to form a transgenic fertilized oocyte; and

allowing the transgenic fertilized oocyte to develop into a transgenic embryo.

2. (Twice amended) The method of claim 1, wherein the [microinserting] co-inserting step is accomplished by piezo-electrically actuated microinjection.

3. (Twice amended) The method of claim 2, wherein the exogenous nucleic acid and the membrane-disrupted sperm head are [microinserted] co-inserted into the cytoplasm of the unfertilized oocyte.

21. (Twice amended) A method for obtaining a transgenic embryo, comprising the steps of:

obtaining a membrane-disrupted sperm head or a demembranated sperm head;

mixing the membrane-disrupted sperm head or demembranated sperm head with an exogenous nucleic acid [containing a desired gene];

[microinserting] co-inserting the mixture into an isolated unfertilized metaphase II oocyte to form a transgenic fertilized oocyte; and

allowing the transgenic fertilized oocyte to develop into a transgenic embryo.